

# Spatiotemporal behavior of T cells in vaccination

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## ABSTRACT

Vaccines are the most cost-effective resource to contain and eliminate infectious diseases. Despite decades of research in the field, several pathogens have eluded the effect of conventional vaccines mostly due their failure in inducing strong T cell responses. There is a need for new vaccine technologies that can surpass this problem. Recent advances in imaging techniques have allowed the study of T cell dynamics within their surrounding cellular niches. This information is invaluable to elucidate the main cellular mechanisms to target in order to optimize vaccine efficiency. In this review, we summarize the most recent key discoveries in T cell behavior in the context of vaccination and immunization.

## 1. Introduction

Vaccines are the most efficient method to control infection diseases and have seriously reduced comorbidity and mortality worldwide. Nevertheless, most conventional licensed vaccines exert protection through antibodies but failed to induce strong T-cell immunity necessary to combat certain intracellular infections. Consequently, diseases such as HIV, malaria and tuberculosis are still causing millions of deaths per year (Foster, 2020). Additionally, endemic virus-related diseases such Influenza A H1N1 and SARS-CoV-2 require vaccines platforms that can be quickly developed and adapted to new mutations, which is difficult to achieve with conventional vaccines. There is also a necessity to move from live-attenuated vaccines to safer alternatives for people with weakened immune system such as the elderly or immunosuppressed patients. Recent research has focused in developing novel adjuvant strategies and antigen-delivery systems with the potential of eliciting robust humoral and cellular protection. These types of vaccines express a recombinant protein or the genetic material to express a piece of the targeted pathogen and are, by themselves, usually poorly immunogenic. Adjuvant strategies are meant to solve this issue by eliciting a transient local inflammation and alert the immune system to create a response against the target antigen.

Advances in vaccine technologies require deep knowledge of the mechanisms underlying T cell differentiation into long-lasting memory cells. Common immunological assays have been crucial to unravel T cell differentiation, but they do not provide information on cell localization and natural behavior that might be key to understand the essential requirements for memory development. Recent advances in cutting-edge

imaging techniques such as multiphoton microscopy (MPM) and multiplex imaging have enabled the quantification of additional parameters during ongoing immune processes while preserving information on cellular microenvironments (Gerner Michael et al., 2012; Medaglia et al., 2017; Stoltzfus et al., 2021; Kuett et al., 2022). To date, due to the difficulty to obtain tissues in humans, most of these techniques have been mainly used in animal models. MPM is based on fluorescence and allows imaging of living tissues. It employs near infrared femtosecond lasers that excite a fluorescent protein or fluorophore by multiple low energy photons arriving simultaneously at the focal plane (Larson, 2011). This phenomenon enhances the ability to image deeper in a tissue with reduced photobleaching and photo toxicity. As such, MPM is the prime imaging method to perform live-imaging of cells in their natural environment, but the number of parameters analyzed at once is limited. Multiplex imaging relies on multiple images taken by confocal microscopy or mass spectrometry and tissue structure and spatial organization are reconstructed in 3 dimensions by computational analyses. Multiplex quantitative imaging can accommodate a high number of parameters to reveal complex spatial relationships between cells, but it relies on fixed samples.

Here, we review recent studies involving T cell spatiotemporal behavior in the context of immunization and vaccination models.

## 2. T cell behavior during priming

T cells survey the whole body looking for their cognate peptide presented by major histocompatibility complex (MHC) molecules at the surface of dendritic cells (DCs). About one to ten T cells in a million can

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recognize a given antigen (Blattman et al., 2002; Jenkins et al., 2010). To enhance the probabilities of a T cell to encounter their cognate peptide the process of T cell activation occurs in specialized tissues named secondary lymphoid organs (SLOs) that include lymph nodes, spleen, Peyer's patches and mucosal tissues, adenoids, and tonsils (Kotsias et al., 2019; Ruddie and Akirav, 2009; Krummel et al., 2016). Vaccination should leverage mechanisms enhancing T cell priming, in particular the key parameters leading to long-term memory differentiation. Thanks to the development of MPM, landmark studies revealed that cluster of differentiation (CD)8 cytotoxic (Mempel et al., 2004) and CD4 helper (Miller et al., 2004) T cell activation occurs in 3 phases, which has been extensively reviewed elsewhere (Bousso, 2008; Qi et al., 2014). The 3 phases of activation have been described only in SLOs so far. During the first phase, T cells scan potential DCs presenting their cognate peptide. This surveillance is optimized by both cell intrinsic (Gérard et al., 2014; Textor et al., 2014) and extrinsic (Bajénoff et al., 2006) mechanisms. This is followed by a second phase where T cells form long-lasting contacts with antigen-bearing DCs. Finally, T cells resume migration and swarm around DCs (Celli et al., 2005). Most imaging studies used models of T cell priming which rely on either subcutaneous DC injection (DC immunization) or priming resident DCs with an antibody that targets the uptake receptor DEC205 coupled to antigen of interest (DEC-Ag). DC immunization is an interesting system that has been leveraged in specific cases of vaccination such as cancer, as it accelerates CD8 T cell memory formation (Badovinac et al., 2005). DEC-Ag is a flexible vaccination strategy that can be used to induce tolerance or immunity depending on absence or presence of adjuvants, respectively (Bonifaz et al., 2002). As such, both models are reminiscent of vaccine strategies currently developed, especially in the context of cancer treatment (van Willigen et al., 2018; Sehgal et al., 2014).

Spatiotemporal characteristics associated with T cell memory differentiation have been identified primarily during the second phase. During DC immunization in the presence of lipopolysaccharide (LPS), CD8 T cells arrest on DCs presenting high, but not low doses of antigen. Arrest of CD8 T cells was necessary for memory formation but irrelevant for the proliferation and development into effector cells (Sehgal et al., 2014). This demonstrates that CD8 T cells start to skew their fate towards memory as early as 24 h after antigen encounter. Requirement of T cell arrest for memory formation has also been suggested following DEC-Ag immunization. DEC-Ag/anti-CD40 immunization of ICAM-1 deficient mice resulted in lack of T cell arrest and inhibition of CD8 T cell memory formation, although the use of full ICAM-1 KO in this system made it unclear whether defect of memory formation was a direct consequence of the lack of arrest (Scholer et al., 2008).

Apart from direct DC-T cell interaction, imaging studies identified another parameter important for memory formation relying on direct T cell communication (Uhl and Gérard, 2020). Following DC/LPS immunization, DEC-Ag/anti-CD40 or antigen/Complete Freund Adjuvant (CFA) immunization, CD4 and CD8 T cells form clusters around DCs (Sabatos et al., 2008; Gérard et al., 2013). Within these clusters, T cells form stable interactions with other T cells in an ICAM-LFA-1 dependent way called synapses. It has been observed that T cells involved in this kind of interactions can exchange cytokines such as IL-2 and IFN- $\gamma$ . T cell communication through IFN- $\gamma$  enhances memory formation, as CD8 T cells that cannot produce or receive IFN- $\gamma$  have a lesser capacity to become memory. This occurs in a specific IFN- $\gamma$  and natural killer rich microenvironment (Krummel et al., 2018), highlighting the role of microenvironment where T cell priming in supporting memory formation.

### 3. The importance of cellular niches organization for T cell dynamics

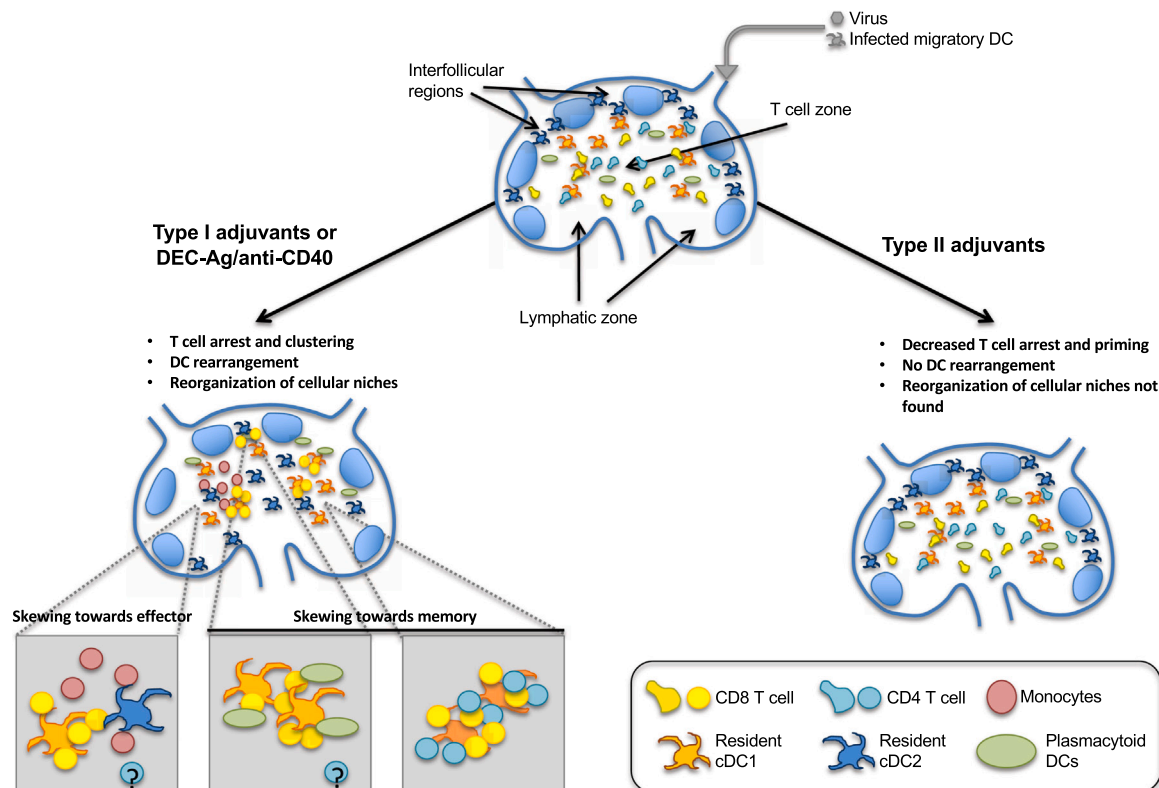
SLOs have a specialized architecture designed to assure that invading pathogens are detected by DCs to initiate an adaptive immune response. According to different phenotypic and functional characteristics, DCs

can be divided into conventional class I DCs (cDC1), conventional class II DCs (cDC2), plasmacytoid DCs (pDCs) and migratory DCs (mDCs), among others (Mildner and Jung, 2014; Eisenbarth, 2019). For the purpose of this review, we will concentrate mostly on cDC1, cDC2 and pDCs. cDC1 have a unique ability to present exogenous pathogen derived-peptides to CD8 T cells while cDC2 prime CD4 T cells more efficiently than cDC1. These differential CD4/CD8 T cell activation potency is related to complex MHC peptide loading mechanisms that are beyond the content of this review and have been extensively described elsewhere (Kotsias et al., 2019; Mildner and Jung, 2014). Finally, pDC is a rare subset that secretes large quantities of pro-inflammatory cytokines such as type 1 interferon (IFNs) upon priming (Bencze et al., 2021). In steady state conditions, cDC1 and cDC2 are differentially distributed (Calabro et al., 2016). In the spleen, cDC1 are primarily located in the T cell zone while cDC2 populate the bridging channels between the red and the white pulp. Similarly, in lymph nodes (LNs), cDC1 are positioned in the deeper paracortex (T cell zone) and cDC2 in the lymphatic sinus (Gerner Michael et al., 2012). This distribution allows cDC2 to be strategically positioned close to circulating particulate antigens that can be too large to access the T cell zone and are critical for inducing CD4 T cell responses (Gerner et al., 2017) (Fig. 1, top picture). Consequently, particulate antigen availability is greater for cDC2 than cDC1 resulting in higher antigen uptake despite having equivalent intrinsic antigen acquisition capability. The limited access to soluble antigen of cDC1 compared to cDC2 might make CD8 T cells particularly sensitive to the original amount of antigen dose inoculated in the periphery. In fact, low doses of antigen were able to prime CD4 but not CD8 T cell in both T cell transgenic and endogenous models, highlighting the importance of antigen biodistribution in vaccine design (Gerner et al., 2017). This information is particularly relevant for vaccine strategies that use antigen encapsulation or conjugation with small nano-materials/protein carriers to target LNs from the periphery independently of migratory DCs (Liu et al., 2014; Jiang et al., 2017; Fries et al., 2021).

Different cellular rearrangements have been observed in response to specific inflammatory stimulus. Type I inflammation such as the one elicited by the adjuvant CpG oligodeoxynucleotides (CpG-ODN), induces a CCR7-dependent resident DC redistribution into the deep paracortex which ultimately enhances the magnitude of CD4 and CD8 T cell responses (Leal et al., 2021). cDC redistribution is also required for CD4 T cell help, which increases the capacity of DCs to promote CD8 T cell memory differentiation (Zhang et al., 2009). T Cell Receptor (TCR) priming of CD4 and CD8 T cells is spatially segregated, occurring on cDC2 and cDC1, respectively. Imaging studies provided evidence that CD4 T cell help then requires antigen transfer to cDC1, which has the capacity to present antigen to both CD4 and CD8 T cells. Following initial TCR priming, CD4 and CD8 T cells relocate and cluster around cDC1, enabling CD4 T cell help (Hor et al., 2015; Eickhoff et al., 2015) (Fig. 1, left picture – bottom 3rd panel). These studies highlight the importance of DC and T cell positioning for memory differentiation.

In addition to DC redistribution, an influx of myeloid cells occurs during inflammatory responses (Leal et al., 2021; Hampton et al., 2015). Type I but not type II inflammatory stimulus induce recruitment of monocytes into the paracortex of the draining LNs (Leal et al., 2021). These monocytes locate in direct proximity to cDC1-CD8 T cell clusters and are the main producer of IL-12, a cytokine crucial for CD8 T cell differentiation (Curtsinger and Mescher, 2010; Zhu et al., 2010). In addition, activated CD8 T cells found in proximity to monocytes-rich areas (Fig. 1, left picture – bottom 1st panel) display a more differentiated effector phenotype, based on t-bet and TCF-1 transcription factor expression, compared to CD8 T cells found in monocytes-poor areas (Leal et al., 2021). These results show that the type of stimulus dictates innate cell recruitment, ultimately skewing T cell differentiation.

Interestingly, T cells reinforce those newly created niches. Using a modified vaccinia Ankara virus model, plasmacytoid DCs (pDCs) migrated into areas of cDC1-CD8 T cell clusters (Fig. 1, left picture –



**Fig. 1. Microenvironments and T cell dynamics during priming.** LNs have a specific architecture with defined microenvironments allowing for rapid response following infection and immunization (top). Type I stimulation/adjuvants (bottom left) lead to the creation of multiple cellular niches specialized in effector or memory differentiation. This is accompanied by T cell re-localization and arrest. Type II stimulation/adjuvants (bottom right), however, does not display similar cellular reorganization and extensive T cell arrest. Whether other types of niches are created is unknown.

bottom 2nd panel), attracted by the chemokines CCL3 and CCL4 produced by activated CD8 T cells (Brewitz et al., 2017). Activated CD8 T cells also secreted the chemokine XCL1, attracting cDC1 cells into pre-formed cDC1-CD8 T cell clusters areas. Disruption of the pDC-cDC1-CD8 T cell axis inhibited CD8 T cell expansion. Mechanistically, pDCs provide IFN-I to cDC1s, which potentiates their maturation and cross-presentation capacities. Indeed, cDC1s lacking IFNAR1 displayed decreased maturation markers, indirectly affecting the magnitude of CD8 T cell response. These results show that activated CD8 T cells orchestrate and reinforce their activating niches.

#### 4. The effect of adjuvants on T cell-polarization dynamics and niche formation

By creating transient inflammatory conditions, vaccine adjuvants elicit the production of cytokines that can modulate the cellular response towards a Th1 or Th2 polarization and can be divided into type I or type II adjuvants, respectively (Korsholm et al., 2010). Most of these cytokine signatures have been directly studied in DCs-adjuvant responders without including other potential cellular contributions from the same microenvironments. In fact, little is known about SLO cellular niches reorganization and T cell spatiotemporal behavior following adjuvanted vaccines. Additionally, different antigens/adjuvants combinations can affect the final T cell polarization elicited highlighting the importance of studying each combination separately. For instance, the Th1-cytokine IFN- $\gamma$  production can be elicited by type II Alum-adjuvanted *Mycobacterium tuberculosis* antigens but not by Alum-adjuvanted ovalbumin (Kool et al., 2012; Safar et al., 2020) indicating the importance of the antigen-adjuvant combination choice when designing a vaccine.

In terms of T cell behavior, adjuvants impact the dynamics of CD4 T

cell polarization (van Panhuys et al., 2014). Type I adjuvant LPS induces increased T cell arrest compared to type II adjuvant papain. However, high antigen concentration overrides adjuvant-induced Th2 polarization by increasing T cell arrest on papain stimulated DCs, leading to Th1 polarization, as detected by IFN- $\gamma$  production (van Panhuys et al., 2014).

The type of adjuvants also impacts DC positioning. Mice immunized subcutaneously or intradermally with type I adjuvants, such as toll-like receptors TLR9, TLR3 and TLR7, exhibit repositioning of resident cDC2 and on less proportion, cDC1 cells, from the peripheral LN regions into the deep T cell zone. Conversely, no clear redistribution was observed with type II adjuvants such as papain or alum (Leal et al., 2021) (Fig. 1, right picture). Overall, this might explain why type II adjuvants are poor inducers of CD8 T cell responses.

#### 5. Conclusion and outlook

There is clear evidence that specialized cellular niches within SLOs are dynamic and crucial for the correct activation of the adaptive immune system (Fig. 1). The emergent quantitative imaging techniques have allowed the characterization of these microenvironments during an immune response and provide critical information about the different cellular niche composition and dynamics during T cell priming. Although multiple studies demonstrated that DC-T cell interactions influence T cell differentiation, it is becoming clear that other cell types are also important for skewing T cell differentiation, and it is as such critical to better understand how T/DC dynamics are integrated within complex cellular niches to regulate memory development.

Most of the knowledge on T cell spatiotemporal behavior comes mainly from studies of widely known infection or antigen models couple with transgenic antigen-specific T cells. These studies, although

valuable, might have discrepancies with endogenous responses. In addition, the route of immunization or infections used for these studies often does not include the most common routes used for licensed vaccines or do not represent the physiological path of infection for pathogens. Those confounding factors make it difficult to extrapolate current information obtained from animal models to develop vaccines for human use. While the direct mouse-to-human translation is not always successful, mouse models are still invaluable to decipher the fundamental mechanisms required to elicit long-term memory responses by directly interfering with pathways of interest in a way which is often not possible in humans. The understanding of those fundamental mechanisms is crucial to efficiently optimize vaccines design and help reduce antigen doses and number of immunizations while increasing efficiency. Another field that has barely been explored is immune dynamics in SLO niches during rechallenge or boost vaccination. Understanding this response is vital to define the ideal timeframe between immunization and boosting and which are the best vaccine combinations to achieve optimal protection.

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